Reproduction by Transverse Fission in Phoronopsis.

By

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With Plate 29.

The occurrence of asexual reproduction in the Phoronidea, though apparently an easy matter to determine, has not proved to be so. Species of Phoronis occur in groups of closely associated individuals or colonies, and it has often been observed that they have the power of casting off and rapidly regenerating the whole of the lophophoral or distal end of the body. Several observers have noted the occurrence, and the process of regeneration has been described in detail by Schultz, who also found that regeneration occurred in both parts of a Phoronis cut in two. Though the naturally detached part can be readily kept under observation, it was never found to develop into a new individual, and this may be the reason why no suggestions were made of the possible reproduction of the animal by simple division of the body. In 1907 Selys-Longchamps (**7**), in repeating the experiments of Schultz, came to the conclusion that natural division of the body probably does occur, and that the animal reproduces in this way. It was not, however, until 1917 that any definite proof of this suggestion was brought forward, when Harmer (**3**), in his rediscovery of Phoronis ovalis, found definite evidence, which seems to place it beyond doubt that such an occurrence does take place.

A species of Phoronis (P. capensis) is very common in

South Africa, but no direct evidence of asexual reproduction has been observed. The colony from which the species was originally described in 1907 was lodged in a piece of limestone. This was suspended in a tank by a piece of copper wire, and, up to the present date, still shows the animals in a flourishing condition; it was suspected therefore that some mode of asexual reproduction does take place, but no proof of this was obtained. Phoronopsis albomaculata was found about the same time (**2**). It has since proved to be not so rare as was then thought, and indeed another species, since recorded from Vancouver Island by Miss Pixell (**5**), seems to occur in abundance. The Cape species is not readily procurable, but has again been found on pieces of limestone in the sea from a depth of 10 to 15 fathoms, one being found at extreme low tide. These stones invariably contained numerous individuals of Phoronis capensis, but only one or two Phoronopsis. All were placed in a well-aërated tank for observation, with a view more especially to obtain some information as to their development, and the occurrence of a possibly interesting larval form, different from that of Phoronis. No further information, however, was obtained on this point, beyond the originally observed occurrence of eggs within the tube, except that the eggs may also be deposited on the rock at some distance from the tube. This was effected by the protrusion of the body to a surprising distance from the tube. The body, during this protrusion, including the lophophoral end, was closely applied to the substratum, and, on two occasions, the eggs were seen to be deposited, and subsequently covered over and attached securely to the rock by a mucous secretion from a large glandular lophophoral organ. The eggs, however, did not develop further, apparently not being fertilised. With a view to overcoming this difficulty, the parts of the large stones containing the animal were cut out and placed together in a suitable vessel, so arranged that the animals could readily be observed by lens or microscope. These were kept under observation throughout a year, but without any further

light being thrown on their development. Another result, however, though not the one looked for, was accidentally obtained. It had been repeatedly observed that, as in other Phoronidea, the lophophoral end of the body was thrown off, and once or twice the actual process, which occupies about half an hour, was observed. For some time after the loss of this region, the rest of the body, which is withdrawn into the tube, is invisible until a new lophophore arises. It was not surprising, therefore, that individuals appeared on stones where they had not been seen before. It was indeed suspected that the cast-off part might develop into a new individual, but no indications of this were detected, though the cast-off portion of the body was carefully watched. On one occasion, however, an undoubted case of reproduction by division of the body was observed, and the details of the process present some interesting features.

In one of the animals kept under observation a constriction appeared round the body about 7 mm. from its free lophophoral end. This became more marked, until a part was ultimately cut off altogether. It moved away from the rest of the body, with periods of rest, and so slowly that its progress could be actually observed only under the microscope. In a few hours it had moved about half an inch. This movement was probably effected by the cilia of the tentacles and body, but may have been assisted by an occasional well-marked bending of the lophophoral end from side to side. There was apparently no adherence to the stone by any mucous secretion, which is often abundant in the disturbed animal. When looked for the following morning the free portion had disappeared, but was ultimately found on the bottom of the vessel under another stone about four inches distant. On the fourth day after division the free portion again divided, below the base of the lophophore, and only a small piece of the body, about 3 mm. in length, remained. Both parts were apparently alive, as the cilia of the tentacles were still active. The lophophoral fragment, which had moved away from the other, began to show indications of a change, as the

tentacles, hitherto straight, commenced to curl up. On the following day no trace of this part could be found anywhere, and it had presumably become disintegrated. The other part, however, was in an apparently healthy condition. Formerly it had been more or less truncate at both ends, but one end now was markedly more pointed than the other, and at times slowly swayed from side to side. It was difficult to keep trace of the animal without running the risk of interfering with its normal course of develpment, and, for this reason also, it was not removed from amongst the stones and sandy mud in the vessel or isolated for closer observation. It continued wandering about for some time, and, when a suitable opportunity was afforded, it was measured, and found to have apparently increased in length somewhat. It was also noted that the circulation, or at least movement of the blood, had again started, as it could be seen through the semi-transparent body, appearing and disappearing at intervals. There was no trace of the lophophore or of the tentacles, but there appeared a small, rounded projection at the smaller end, which seemed to be the epistome. That this projection was ciliated was apparent from the fact that a current of water with contained particles was observed to flow towards it.

On the following day, the tenth after the first division, an opportunity was afforded of observing the other end of the animal, as it had penetrated into a small heap of muddy débris, leaving its posterior part exposed. This presented a somewhat swollen appearance, which, however, varied fairly rapidly, being sometimes almost globular in outline. This movement was well marked. The transverse corrugations, characteristic of the general surface of the body, were continued on to the swollen part, except at its centre posteriorly. Here a small, circular area was devoid of corrugations, and somewhat whitish in colour, but not raised above the general rounded surface of the body. The animal gradually penetrated into the débris, and became nearly hidden in a small crevice of the stone.

This was supposed to be the beginning of the tube-formation and the sedentary life, but on the following day the animal was found to have moved off again, being found under a stone a few inches away. The movements were now much more definite, the anterior end turning readily to any loose material and penetrating it; occasionally also the body in progression became much elongated. Its rate of progression was noted, and it was found on occasions to pass over a distance equal to the length of its body in about two minutes. The rate of movement, however, was not regular, and it frequently ceased completely, the anterior end meanwhile moving about from side to side. The most marked feature, however, of this stage was the appearance of a knob-like projection at the posterior end, at the place occupied by the whitish spot already mentioned. It was of a clear but not homogeneous appearance, as, internally, it appeared to have convoluted strands or corrugations.

The animal could not be found on the following day without the risk of undue disturbance and probable injury, but it was again seen next morning, and two new features were observed, namely, the appearance of a low but distinct ridge round the anterior projection. As subsequent development showed, this was the beginning of the lophophore. A second and more striking change was the growth of the posterior prominence, which had now become about half a millimetre in length (Pl. 29, fig. 1).

On the following day the animal was found with difficulty and only after all the stones had been removed from the tank. Its presence was detected by a slight protrusion of the regenerating lophophoral region from a small heap of mud and sand. This was carefully cleared away, when it was found that the animal was no longer free, but was securely fixed to the bottom of the tank by the pedunculate posterior projection. The whole of this part, but more especially its terminal portion, was covered with a very adhesive mucus, which adhered to the surrounding particles of sand, etc., as well as to the substratum. Apparently the animal was

at a critical stage in its development, and it was therefore detached from its point of adhesion and disentangled from the surrounding material. It was then examined microscopically in a watch-glass full of water, and the following particulars observed. The total length of the body, including posterior appendage, was 2·76 mm., of which the body was 1·9 mm., the appendage 0·86 mm. (Pl. 29, fig. 2). The length of the body, however, varied with the movement of the animal, but was decidedly shorter though thicker than in the previously observed stages. This may have been due to the irritation of the animal on removal to new conditions. The lophophore (Pl. 29, fig. 2, *l.*) was more defined, the tentacles being marked out as lobular protrusions of the lophophoral ridge. The lophophore-opening was well marked, and through it could be seen a distinct prominence, apparently the epistome (Pl. 29, fig. 2, *epist.*) The body became somewhat narrower below this region, and widened out greatly towards its aboral end. An indistinct white line could be traced along the left side—apparently the nerve-cord. At its aboral extremity the body was very rounded, and a slight indentation appeared where it joined the appendage. This tail-like appendage, which for convenience may be referred to as the peduncle, is of more particular interest. In the living animal it was observed to be about one-third of the total length. It did not merge gradually into the body, its point of attachment being situated in the centre of a depression, so that in a lateral view the point of junction was not observed. The other or free end appeared to be irregular in shape, but was partly hidden by the very adhesive mucus and entangled particles which surrounded it. The peduncle was of a slightly yellow colour, like that of the animal generally, and was irregular in outline, exhibiting three or four protrusions on each side. Its structure, as seen by transmitted light under the microscope, was not homogeneous. The protrusions were very transparent and homogeneous, but the main part appeared to have a few thick strands running lengthwise. This appearance was not due to strands of internal tissue,

but to epidermal foldings, as was shown in sections (Pl. 29, figs. 8–13). The secretion of the mucus appeared to be confined chiefly to the distal or free end of the peduncle. There was little or no contraction of this part, though it was well marked in the body of the animal.

As the animal at this stage appeared to be about to assume the normal tubicolous habits, and as there was some risk of losing sight of it altogether, it was thought best to preserve it for minute examination by sections after removing such particles as might interfere with cutting. It was accordingly fixed in sublimate with 5 per cent. acetic acid, and stained in alum carmine.

The various stages of the transformation may be briefly recapitulated:

March 21st	1st day	Division from parent.
„ 22nd	2nd „	Crawling movement.
„ 23rd	3rd „	„ „
„ 24th	4th „	Second division; lophophoral region cast off.
„ 25th	5th „	Anterior end became narrower.
„ 26th	6th „	Not seen.
„ 27th	7th „	„
„ 28th	8th „	„
„ 29th	9th „	Indication of epistome; circulation re-started.
„ 30th	10th „	Posterior end rounded, with white patch.
„ 31st	11th „	Projection at posterior end.
April 1st	12th „	Not seen.
„ 2nd	13th „	Appearance of lophophore and elongation of posterior projection to ·5 mm.
„ 3rd	14th „	Further elongation of posterior projection and fixation of animal by a mucous secretion at its free end.

The process of regeneration occurring meanwhile in the parent animal may be noted here. This was at the normal rate observed in other cases, and much more rapid than in the detached portion. On the first day it remained completely withdrawn into its tube, but was visible as a truncate projection on the second day, and on the ninth day the tentacles of the lophophore could be made out, being at the stage only reached by the detached part on the fourteenth day, on which latter date the tentacles of the parent had grown out to a length of about 1 mm.

No other case of natural division and growth of a detached part was observed, and as it was desirable to obtain other stages for further examination, the experiment was made of cutting off the protruding parts of normally growing individuals and observing their subsequent changes. About a dozen in all were so treated, a part of the body being cut off about equal in length to that observed in the spontaneous division. Various results followed which need not be detailed, as in all except two cases the part cut off became disintegrated. In one case the piece again divided, but subsequently disappeared; in another, however, after the second division development was seen to proceed, as in the case of natural proliferation. In this case a larger piece of about 22 mm. was cut off. This was on April 8th. On the 9th it was found not far off, but reversed in position. On the 10th a constriction appeared about a millimetre from the base of the lophophore, and on the same day a slight protuberance was seen at the aboral end, measuring about ·24 mm. in breadth and ·15 mm. in length. Special note was made of this part. Internally it was of a whitish colour, the outer parts being of a yellowish colour, similar to that of the body generally. It was not seen again until the 12th, when it had considerably advanced in development. A collar-like ridge, representing the beginning of the lophophore, was seen, and projecting from its centre was a conspicuous prominence, which apparently represented the early appearance of an epistome, as in the previous case. The posterior projection

was more pronounced. It will be observed here that the times and order of the various changes were different from those in the first case. Thus the second division was on the third day in place of the fourth, and the posterior prominence appeared much earlier. The fragment was also much less active, and did not move about freely. As it did not look very lively, and no movement of blood was observed, it was preserved for longitudinal sections with a view specially of ascertaining the mode of origin of the posterior projection.

The following is the record of the changes with their dates :

April 8th . First (artificial) division.
„ 9th . Unaltered, but removed a short distance.
„ 10th . Second (spontaneous) division, and appearance of posterior projection.
„ 12th . Epistome and rudiment of lophophore.

These observations are given in some detail as they serve to show that transverse division may be a normal mode of reproduction in Phoronopsis and yet not be readily observed even under favourable conditions, and in spite of the fact that this animal lives in isolated tubes which do not penetrate the substratum. The difficulties of observation in the case of species of Phoronis forming colonies would be greater, but a somewhat similar process to that above described may occur in this genus, and it would be well worth while to examine by sectioning the structure of the fragments of the animal said to be found in such colonies, more especially their aboral extremities.

Minute Structure of Free Part.

For convenience the piece set free by natural division of the animal may be referred to as A, of which transverse sections were made ; the second, obtained by artificial division, may be designated B, of which longitudinal sections were made.

Some features are worthy of note in the process of

regeneration of the oral region of the body, but as we are chiefly concerned here with the changes which take place in the aboral region, the origin and structure of the peduncle may first be considered. In specimen B (Pl. 29, fig. 3) the beginning of the change is clearly seen. The ruptured aboral ends of the ascending and descending parts of the alimentary tract have closed up and become somewhat pointed at their extremities, which are close to each other, but there is, as yet, no connection between their cavities. Just above their extremities and almost completely encircled by them is a large space occupied by blood (Pl. 29, fig. 3, *bl.*), and from this both afferent and efferent parts of the vascular system can be traced, so that free circulation is already possible. Lying below this, and immediately over the aboral projection, is a lenticular and compact mass of cells of a well-defined nature (Pl. 29, fig. 3, *cœl. ep.*) ; the nucleus of each cell is comparatively large, and the body is drawn out into two long, tapering ends. The mass of cells is not at this point directly connected with the blood-vessels, but other sections further off show that there are fine blood-vessels, often of the diameter of one blood-corpuscle, connecting it with the main vascular system. As to the origin of these cells, there seems to be little doubt from their general appearance, and from the fact that they can be traced on each side to the layer of cells lining the wall of the body-cavity, that they constitute a mass of proliferating cells of the cœlomic epithelium. Below this mass and completely in the aboral projection these same cells are found, not in close contact with each other, but forming a sort of loose network. There are as yet no other kinds of cells in the cavity of the projection.

The outer walls of the projection are a continuation of the single-celled layer of epidermal cells of the body generally, and of the well-developed basement-tissue (Pl. 29, fig. 3, *b.t.*). These regenerating cells, however, are smaller than those of the normal epithelial cells, and not so well defined, though a few glandular cells may be seen among them.

In the median section figured there were no traces of the foldings of the epithelium so characteristic of a later stage, but in more lateral sections the commencements of these were indicated.

The muscular layers of the body-wall cease abruptly near the edge of the protuberance, and are not continued into its cavity; the same is true also of the nerve-cord.

The further development of the projection was seen in the fragment A, in which it attained a length of ·86 mm. in four days. The ends of the alimentary tract are not yet joined, though that of the descending limb has become enlarged and sends out diverticula (Pl. 29, fig. 6), between which blood-vessels may be seen in some sections. The joined ends of the vascular system are as before, but there is an increase of small ramifying branches. Below this is again to be found a mass of the characteristic elongate cells, which were found to originate from the cœlomic epithelium. These extend into the lumen of the peduncle, but in a somewhat modified condition; they are less definite, and scattered throughout them are small globular particles (Pl. 29, fig. 9, *ft.*), apparently of nourishing material, for they closely resemble the fatty globules seen in the vaso-peritoneal epithelium of the body-cavity. Cori (**1**) has described these, and Ikeda (**4**) has shown that they arise in connection with a proliferation of the cœlomic epithelium covering the blood-vessels, and serve as nourishment for the developing gonads; they probably serve here as nourishment for the growing peduncle. Miss Pixell (**5**) describes and figures them in the genus Phoronopsis.

A conspicuous feature of the inner tissue of the peduncle is also muscular tissue, varying from thick strands like the longitudinal muscles of the body to thin elements similar to the radial muscles. A few elongate cells lying immediately beneath the epithelium in some sections may represent the beginnings of the circular muscles. The epidermal elements were similar to those already noted in the fragment B, but were smaller, and there was no trace of the thick basement-tissue. There were amongst the cells numerous clear

glandular cells, and the peripheral parts, more especially of the cells at the free end, contained fine yellow granular material.

A conspicuous feature of the epidermis was the manner in which it was folded in some places, more particularly at the beginning and the middle (Pl. 29, figs. 7–12). This folding was carried to such an extent in places that there was no lumen in the peduncle (Pl. 29, fig. 12). That these foldings were not due to shrinking in the preservative is evident from the fact that they were seen in the living condition as thick strand-like structures in the semi-transparent tissue of the peduncle.

Some features in the origin of the epistome are worthy of note. At an early stage in the regeneration of the oral end of the body there appeared between the ruptured ends of the ascending and descending limbs of the alimentary tract a conspicuous conical prominence (Pl. 29, figs. 1 and 4, *epist.*). It first appears shortly before the ridge of tissue which will form the lophophore, but as development proceeds it is not so well marked. It still appears in section as a prominent organ, but has apparently shrunk in the preservative. In life it may contain a space, but in the sections it is seen that the whole of its interior is solid. At the base of the organ is a mass of tissue resting on a large cavity filled with blood. This tissue (Pl. 29, fig. 4, *cœl. ep.*) is in close contact with the blood and also with the regenerating mouth and anus, which have not as yet appeared. It appears to be a mass of proliferating cells of the cœlomic epithelium, similar to that observed at the aboral end of the body.

It may be suggested that the comparatively large structure here referred to, though arising between the mouth and anus, is not the homologne of the epistome. This may possibly prove to be so when the further stages are known.

Significance of the Mode of Development of Peduncle.

The peduncle, after its first appearance, is formed by a rapid multiplication of the cells of the single-layered

epidermis, which is thus thrown into a number of folds or involutions, and its apparently rapid growth and elongation in subsequent stages (·36 mm. during the last day) is probably due to the unfolding or eversion of these. This is a process which at once recalls a somewhat similar phenomenon in the larval development, namely, the rapid proliferation of the epidermal cells at a point in the body and the consequent involution of the epidermis, which, later on, becomes somewhat suddenly everted, and into which the alimentary tract penetrates. The later stages of the peduncle are not known, but it seems to be probable that here also the lumen of its folded walls will be dilated to receive the growing visceral elements of the body. In other words, the process of regeneration is here a repetition, or at least a recapitulation, of the process of ordinary development from the ovum.

Summary.

(1) Phoronopsis has been observed to reproduce asexually by transverse division of the body.

(2) The division occurs in the muscular region of the body.

(3) The detached part is capable of locomotion, and divides a second time below the lophophore, which is thrown off and disintegrates.

(4) The remaining part, after moving about freely, develops an anterior projection (epistome ?), a lophophoral ridge, and later an aboral projection.

(5) The epidermis of the aboral projection is thrown into a number of folds or involutions, by the unfolding of which it somewhat suddenly increases in length at later stages and assumes the form of a peduncle.

(6) The animal then becomes fixed by a mucous secretion at the free end of this peduncle.

(7) The whole process, from first division to pedunculate fixed form, occupied fourteen days.

(8) The peduncle consists externally of a proliferation of

the epidermis of the body and internally of modified cells of the cœlomic epithelium, fatty particles and muscular elements.

References.

1. Cori, C. J.—"Untersuchungen über die Anatomie und Histologie der Gattung Phoronis," 'Zeit. f. wiss. Zool.,' Bd. li, 1890. p. 480.
2. Gilchrist, J. D. F.—"New Forms of the Hemichordata from S. Africa," 'Trans. S. Afr. Phil. Soc.,' vol. xvii, 1907, p. 151.
3. Harmer, S. F.—"On Phoronis ovalis, Strethill Wright," 'Quart. Journ. Micr. Sci.,' vol. 62, 1917, p. 115.
4. Ikeda, I.—"On the Development of the Sexual Organs and their Products in Phoronis," 'Annot. Zool. Jap.,' vol. iv, 1903, p. 141.
5. Pixell, H. L. M.—"Two New Species of the Phoronidea from Vancouver Island," 'Quart. Journ. Micr. Sci.,' vol. 58, 1912, p. 257.
6. Selys-Longchamps, M. de.—"Ueber Phoronis und Actinotrocha bei Helgoland,' 'Wiss. Meeresunt. Kiel u. Helgoland,' N.F., Bd. vi. Abt. Helgoland, Heft 1, 1903.
7. ——— "Phoronis," 'Fauna u. Flora G. v. Neapel,' 30 Monogr., 1907.

EXPLANATION OF PLATE 29.

Illustrating Dr. J. D. F. Gilchrist's paper on "Reproduction by Transverse Fission in Phoronopsis."

The magnification of figs. 1 and 2 is indicated by the scale accompanying fig. 1, that of the other figures by the scale accompanying fig. 3. All the figures refer to Phoronopsis albomaculata, Gilchrist.

List of Abbreviations.

al. c. Alimentary canal, descending part. *c.m.* Circular muscles. *al. c'.* Ascending part. *bl.* Blood-corpuscles. *b. t.* Basement-tissue. *cœl. ep.* Cœlomic epithelium. *d.* Débris. *ep.* Epidermis. *epist.* Epistome. *f. t.* Fatty tissue. *l.* Lophophore. *l. m.* Longitudinal muscles. *n.* Nerve. *ped.* Peduncle.

Fig. 1.—Free crawling form of Phoronopsis, produced by two transverse divisions of the body of the adult, thirteen days after first division.

Fig. 2.—Pedunculate fixed form, fourteen days after first division.

Fig. 3.—Sagittal section of specimen B (free form), showing commencement of posterior projection.

Fig. 4.—Sagittal section of oral extremity of specimen B.

Fig. 5.—Transverse section of body of specimen A (pedunculate attached form) near the extremities of the digestive tract, at the point of junction of the afferent and efferent blood-vessels.

Fig. 6.—Transverse (somewhat oblique) section of aboral end of body, showing two diverticula of the extremity of the descending part of the alimentary canal, and the position of the cœlomic epithelium which marks the origin of the peduncle.

Fig. 7.—Transverse section of attached form, showing mode of origin of the peduncle from the body.

Fig. 8.—Transverse section of proximal end of peduncle, free from body.

Figs. 9–11.—Transverse sections of peduncle, showing nature of its contained tissue.

Fig. 12.—Transverse section of peduncle, showing excessive foldings of epidermis and obliteration of central space.

[Sections 7–12 are at about regular intervals in the first half of the peduncle.]

Fig. 13.—Section of distal and more rounded part of peduncle, showing particles entangled in mucus secreted by this part. The central tissue is omitted.